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Serum leukotrienes, circulating neutrophils, and high sensitivity C-reactive protein in Chinese children with sleep-disordered breathing

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ABSTRACT

Background: A number of previous works have shown that leukotriene (LT) concentration emerged disease severity-dependent increases in both exhaled breath condensate and urine of sleep-disordered breathing (SDB) patients. However, few studies have investigated how circulating level of LTs contributes to systemic inflammation of SDB, and the relationship between LT production, leukocyte count and high sensitivity C-reactive protein (hsCRP) level in SDB disease remains controversial.
Methods: Prospective, observational study that included standard questionnaires, physical examinations and polysomnography. Serum leukotriene B_4 (LTB_4) and cysteinyl leukotrienes (cysLTs) were determined by means of enzyme-linked immunosorbent assays.
Results: A total of 166 children with SDB and 45 control subjects were recruited. SDB children had increased serum levels for both LTB_4 and cysLTs as well as neutrophil (Neu) count and hsCRP than the control group, and all the inflammatory parameters emerged disease severity-dependent increases. LT production correlated significantly with Neu count and hsCRP level. In the regression model, both apnea–hypopnea index and body mass index z-score were significant predictors of LTB_4 and cysLTs ($p < 0.001$).
Conclusions: The activated systemic inflammatory response as reflected by serum elevations of LTs, Neu counts and hsCRP is present in children with SDB, and the magnitude of inflammation emerged disease severity-dependent. The level of LTs is significantly associated with circulating Neu counts and hsCRP values in SDB.

1. Introduction

Sleep-disordered breathing (SDB) is a highly prevalent pediatric disorder, affecting 4% to 11% of children all over the world [1]. The etiology and pathophysiological mechanisms leading to SDB in children have not been clearly elucidated, but may include a complex interplay between anatomic factors (mainly adenotonsillar hypertrophy), neuromuscular weakness and an underlying genetic predisposition toward the disease [2]. Recently, evidence has emerged linking the presence of systemic inflammation to the pathophysiology of SDB [3–5].

Among the major inflammatory mediators, leukotrienes (LTs) are a family of inflammatory lipid mediators, which includes leukotriene B_4 (LTB_4) and $LTC_4/D_4/E_4$ (cysteinyl leukotrienes, cysLTs). A number of previous works have shown that LT concentration emerged disease severity-dependent increases in both exhaled breath condensate [6] and urine [6,7] of SDB patients. And it is speculated that LTs may participate in the pathogenesis of SDB by recruiting inflammatory cells, promoting mucus production, producing smooth muscle contraction and inducing vascular damage [8,9]. However, few studies have investigated how circulating level of LTs contributes to systemic inflammation of SDB. Neutrophils are wildly considered as a vanguard of the acute innate immune response to invading pathogens [10]. However, uncontrolled release of their formidable array of toxic substances may inflict damage to surrounding tissues and propagate inflammatory responses [11]. Neutrophilic systemic inflammation has been shown in adult patients with SDB [12], but no studies have investigated the circulating neutrophil level in SDB children. High sensitivity C-reactive protein (hsCRP) is believed to be both a by-product and a mediator of the low-grade inflammation that occurs in atherosclerosis, making it a useful marker

Abbreviations: AHI, apnea–hypopnea index; Bas, basophil; BMI, body mass index; CysLTs, cysteinyl leukotrienes; DBP, diastolic blood pressure; Eos, eosinophil; FLAP, 5-lipoxygenase activating protein; HsCRP, high sensitivity C-reactive protein; HDL, high-density lipoprotein; Hgb, hemoglobin; IL, interleukin; IQR, interquartile range; LDL, low-density lipoprotein; 5-LO, 5-lipoxygenase; LTs, leukotrienes; LTB_4, leukotriene B_4; LTC_4, leukotriene C_4; LTD_4, leukotriene D_4; LTE_4, leukotriene E_4; Lym, lymphocyte; MAP, mean arterial pressure; Mon, monocyte; Neu, neutrophil; OAI, obstructive apnea index; ODI, oxygen desaturation index; PLT, platelet count; PSG, polysomnography; RBC, red blood cell count; SBP, systolic blood pressure; SDB, sleep-disordered breathing; SE, sleep efficiency; SLT90%, percentage of time spent saturation lower than 90%; SpO_2, pulse oximetric saturation; T&A, tonsillectomy and adenoidectomy; TST, total sleep time; WBC, white blood cell count.

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for cardiovascular risk stratification [13,14]. An association between SDB and the development of cardiovascular diseases has been suggested for several years, and intermittent hypoxia/reoxygenation is likely to play a significant role in the formation of cardiovascular morbidity [15]. However, the relationship of hsCRP level and SDB remains controversial.

Based on such considerations, we examined the relative amount of LTs, leukocyte count and hsCRP in serum to evaluate the magnitude of systemic inflammation. Our objective was to investigate the relationship of circulating LT production with severity of SDB, leukocytes and hsCRP level in children.

2. Methods

2.1. Participants

The study protocol was approved by the Beijing Children's Hospital Ethics Committee, and informed written consent was obtained at the beginning of the assessment from all the parents and the children >6 years of age.

Children consecutively referred to the Sleep Center from January 2012 to May 2012 for polysomnography (PSG) because of suspected SDB were enrolled in the study. At the same time, age-, sex-, and weight-matched children with heterotropia or blepharoptosis but without a history of snoring were recruited from the Ophthalmology Departments as control subjects. Inclusion criteria were the presence of habitual snoring (snoring as reported by parents >3 nights/week and last >3 months) and age from 2 to 9 years. Exclusion criteria for both snoring patients and controls included the presence of cardiovascular, neuromuscular, craniofacial or genetic disorders; allergic disorders; acute or chronic inflammation; use of corticosteroids, antibiotics or LTs modifier within 1 month; and previous adenotonsillectomy.

2.2. Clinical evaluation

A detailed questionnaire and physical examination were given. The questions referred to sleep/physical/emotional/daytime symptoms, presence of comorbidity, past medical history, medication use and family history. Weight and standing height were measured, and the body mass index (BMI) was then calculated as weight (in kilograms)/height (in square meters). We transformed an individual child's BMI value to a z-score based on the gender-specific and age-specific reference values for Chinese children [16]. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured with a sphygmomanometer on the upper arm using standard techniques. Tonsil size was graded from 1 to 4 by direct inspection of the oropharynx [17]. Adenoid size, determined by nasopharyngoscopy, was graded from 1 to 4 in accordance with Modrzynski and Zawisza's criteria [18]. The sum of the adenoid and tonsil scores was used as the global estimate [19].

2.3. Polysomnography

A standard overnight PSG (Compumedics E-series; Compumedics Inc; Abbotsford, VIC, Australia) was performed in the Sleep Center at Beijing Children's Hospital for all children with snoring except the control subjects. No sleep deprivation or sedation was used. Patients were studied for at least 8 hours in a dedicated, quiet and dark room with an ambient temperature of 24 °C in the company of one of their parents. The following parameters were measured: four-channel electroencephalogram with bilateral central and occipital leads, submental electromyogram, electrooculogram, electrocardiogram and body position. The respiratory variables included thoracic and abdominal wall movement, nasal airflow and pulse oxygen saturation. Standard techniques were used to analyze the polysomnogram by an experienced sleep technologist who was unaware of the clinical findings. Sleep staging was assessed using the criteria of Rechtschaffen and Kales [20]. Sleep efficiency (SE) refers to the percentage of the total recording time that the patient was asleep. Obstructive apnea was defined as the absence of airflow with persistent chest wall and abdominal movement for more than two breaths in duration [21,22]. Hypopnea was defined as a reduction in oronasal flow of at least 50% compared to baseline with a corresponding decrease in pulse oximetric saturation (SpO_2) of at least 4% and/or arousal [21]. The apnea–hypopnea index (AHI) was defined as the average number of apneas and hypopneas per hour of total sleep time (TST). The obstructive apnea index (OAI) was defined as the average number of apneas per hour of sleep. The oxygen desaturation index (ODI) was defined as the number of oxyhemoglobin desaturation events per hour of sleep at desaturation of at least 4% from baseline. Sleep staging and respiratory events analysis was summarized by computer software (ProFusion 2; Compumedics Inc). SDB severity was classified by AHI. Briefly, mild SDB was those with AHI <5 episodes/h and ≥1 episodes/h, moderate SDB was those with AHI <20 episodes/h and ≥5 episodes/h, and severe SDB was those with AHI ≥20 episodes/h.

2.4. Blood samples

Venous blood was collected between 7:00 and 8:00 a.m. from snoring children after overnight PSG and the control subjects. Full blood counts (white blood cell count [WBC], neutrophil [Neu], lymphocyte [Lym], monocyte [Mon], eosinophil [Eos] and basophil [Bas] count) as well as blood biochemistry including glucose, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides were measured. HsCRP levels were quantified by a high-sensitivity immunonephelometric method with a lowest detection limit of 0.08 mg/L (N High Sensitivity CRP; Olympus Diagnostica GmbH).

2.5. LT ELISAs

The blood samples were centrifuged at 2500 rpm for 10 minutes at 4 °C immediately after venipuncture, and sera were then separated and stored at −80 °C until assay. Two specific commercially available enzyme-linked immunoassay kits (Leukotriene B_4 EIA kit and Cysteinyl Leukotriene Express EIA kit; Cayman Chemical Company; Ann Arbor, MI, USA) were used for quantification of LTB_4 and cysLTs in the serum following the manufacturer's instructions. All samples were loaded in duplicates and assayed in at least two dilutions, and plate reader absorbance results were analyzed with a four-parameter logistic curve fit. The intra-assay and inter-assay variabilities for LTB_4 assays were 8.37%–17.64% and 7.78%–21.68% respectively, and those for cysLTs assays were 5.6%–9.2% and 6.1%–16.5% respectively. The specificity for LTs was 100% (except for LTE_4, which was 67%). The detection limit of the assays was 21.3 pg/ml for LTB_4 and 6.7 pg/ml for cysLTs. All measurements were completed in 1 day using stored sera to avoid interassay variability.

2.6. Data analysis

Data were presented as mean ± SD or median (interquartile range, IQR) depending on the distribution unless stated otherwise. PSG indices, hsCRP values and LT concentrations were log-transformed (natural logarithm) and SpO_2 was logit-transformed to correct for skewed distribution. The ODI and percentage of time spent saturation lower than 90% (SLT90%) were assigned as 0.01 if the original data were 0 when transformed. One-way analysis of variance followed by post hoc tests for pair comparisons (Bonferroni test) was used for continuous variables and the χ^2 test (Yates correction) for categorical characteristics. If data could not be transformed to approach normal distribution, a Kruskal–Wallis test was applied. Correlations were analyzed without adjustment by using Pearson correlation. Partial correlation was applied to assess the linear association of LT concentration with other systemic inflammatory markers after adjustment for covariates including age, sex, BMI z-score, Neu, Lym and Mon. Stepwise multiple linear regressions

were performed to identify independent predictors of LT production. All statistical analyses were conducted using statistical software (version 16.0; SPSS, Chicago, IL, USA). A two-sided p value of <0.05 was considered statistically significant.

3. Results

The 242 children who fulfilled the inclusion criteria were recruited, of which 31 subjects were excluded because of blood sample hemolysis ($n = 11$), incomplete clinical data ($n = 10$), or refusal to undergo the PSG ($n = 10$). Ultimately, a total of 211 subjects were included in the analysis.

3.1. Patient data

The characteristics of involved subjects stratified by SDB severity are given in Table 1. There was no statistical difference between the groups with respect to age, sex, disease course, adenotonsillar size, blood pressure or metabolic variables. However, BMI z-score was higher in SDB groups than the controls. As expected, AHI, OAI, ODI, and SLT90% increased and SpO_2 decreased progressively with the severity of the disease.

3.2. Biomarker levels

The four study groups differed significantly regarding LT concentration, full blood count and hsCRP ($p < 0.001$). SDB patients demonstrated higher levels for both LTB_4 and cysLTs than control subjects, and LT production showed disease severity-dependent increases (Table 1). Significantly higher Neu counts and hsCRP levels were also detected in the serum of the SDB group than the controls (Table 1), suggesting an increased basal systemic inflammatory state.

3.3. Effects of Confounders on LTs Concentration

By Pearson correlation, log-transformed LT concentrations were positively correlated with BMI z-score ($\ln LTB_4$: $r = 0.358$, $p < 0.001$; lnCysLTs: $r = 0.383$, $p < 0.001$) and mean arterial pressure (MAP) ($\ln LTB_4$: $r = 0.229$, $p = 0.001$; lnCysLTs: $r = 0.204$, $p = 0.003$), but not with age, sex, disease duration, adenotonsillar size or metabolic variables (Table 2).

After adjustment for age, sex and BMI z-score, there was a significant correlation between LT production and other systemic markers including Neu ($\ln LTB_4$: $r = 0.299$, $p < 0.001$; lnCysLTs: $r = 0.202$, $p = 0.003$) and lnCRP ($\ln LTB_4$: $r = 0.343$, $p < 0.001$; lnCysLTs: $r = 0.370$, $p < 0.001$) (Table 3).

Table 1
Characteristics of recruited subjects stratified by SDB severity.[a]

Variables	Control ($n = 45$)	Subjects with SDB		
		Mild SDB ($n = 50$)	Moderate SDB ($n = 64$)	Severe SDB ($n = 52$)
Age, yrs	5.06 ± 1.64	5.59 ± 1.75	5.16 ± 1.63	5.63 ± 1.85
Male/Female ratio	27:18	35:15	39:25	39:13
BMI z-score	0.28 ± 0.85	0.54 ± 1.11	0.97 ± 1.45*	1.08 ± 1.45*
Clinical data				
Disease duration, yrs	–	2.0 (0.9, 3.3)	2.0 (1.0, 3.0)	2.0 (1.0, 3.0)
Adenotonsillar size score	–	5.5 (5.0, 6.0)	6.0 (5.0, 6.0)	6.0 (5.0, 7.0)
SBP, mm Hg	91.04 ± 11.18	95.26 ± 15.05	96.52 ± 16.96	102.13 ± 19.93
DBP, mm Hg	58.84 ± 9.19	62.20 ± 9.84	62.81 ± 10.12	65.02 ± 10.78
MAP, mm Hg	69.58 ± 9.52	73.22 ± 11.44	74.05 ± 12.13	77.39 ± 13.64
PSG parameters				
SE, %	–	83.72 ± 8.37	79.71 ± 9.88	82.29 ± 10.51
AHI, episodes/h	–	3.05 (1.80, 4.13)**	10.50 (7.70, 15.58)**	32.80 (28.03, 52.40)**
OAI, episodes/h	–	1.10 (0.60, 1.50)**	4.00 (2.00, 6.90)**	11.35 (5.95, 18.10)**
ODI, episodes/h	–	0.90 (0.30, 1.48)**	6.10 (2.40, 10.08)**	25.70 (16.10, 46.95)**
Mean SpO_2, %	–	98.0 (96.4, 98.5)**	97.0 (94.30, 97.5)**	95.6 (92.5, 96.8)**
Minimal SpO_2, %	–	91.0 (87.0, 93.3)**	85.0 (81.0, 88.8)**	72.5 (52.3, 79.5)**
SLT90%, %TST	–	0.0 (0.0, 0.0)**	0.1 (0.0, 0.7)**	4.2 (1.2, 12.0)**
Hematologic parameters				
Glucose, mmol/L	4.74 ± 0.49	4.58 ± 0.52	4.67 ± 0.41	4.50 ± 0.56
Total cholesterol, mmol/L	4.15 ± 0.67	4.16 ± 0.65	4.32 ± 0.68	4.31 ± 0.82
Triglyceride, mmol/L	0.69 (0.59, 0.85)	0.61 (0.51, 0.79)	0.61 (0.51, 0.71)	0.69 (0.54, 0.940)
HDL, mmol/L	1.40 ± 0.29	1.24 ± 0.25	1.33 ± 0.32	1.27 ± 0.31
LDL, mmol/L	2.39 ± 0.62	2.58 ± 0.56	2.68 ± 0.61	2.65 ± 0.62
WBC, $\times 10^9$/L	6.74 ± 1.50	6.54 ± 1.25	7.31 ± 1.55***	7.38 ± 1.67*,***
Neutrophils, $\times 10^9$/L	2.79 ± 0.95**	3.30 ± 1.04	3.75 ± 1.24	4.05 ± 1.41***,****
Lymphocytes, $\times 10^9$/L	3.29 ± 1.03**	2.69 ± 0.75	2.93 ± 0.83	2.68 ± 0.72
Monocytes, $\times 10^9$/L	0.42 ± 0.15	0.39 ± 0.11	0.44 ± 0.18	0.47 ± 0.18
Eosinophils, $\times 10^9$/L	0.16 (0.09, 0.23)**	0.09 (0.05, 0.19)	0.12 (0.07, 0.19)	0.10 (0.06, 0.19)
Basophils, $\times 10^9$/L	0.04 (0.03, 0.06)	0.04 (0.03, 0.06)	0.05 (0.03, 0.06)	0.04 (0.03, 0.05)
RBC, $\times 10^{12}$/L	4.60 ± 0.35	4.53 ± 0.27	4.51 ± 0.50	4.64 ± 0.53
Hgb, g/L	127.38 ± 6.97	128.98 ± 8.92	125.73 ± 13.80	125.85 ± 20.02
PLT, $\times 10^9$/L	277.02 ± 54.61	299.86 ± 62.62	290.05 ± 64.30	297.88 ± 64.88
LTB_4, pg/ml[b]	65.37 (39.23, 78.17) **	95.74 (67.15, 151.00)	131.87 (75.29, 198.38)	215.99 (150.49, 279.68)***,****
CysLTs, pg/ml[b]	169.46 (137.13, 249.52)**	286.32 (184.63, 377.36)**	420.62 (258.89, 623.00)**	639.60 (393.90, 816.73)**
HsCRP, mg/L[b]	0.14 (0.11, 0.28)**	0.29 (0.11, 0.70)	0.32 (0.12, 0.75)	0.70 (0.41, 1.70)***,****

[a] Values represent mean ± SD or median (IQR) depending on the data distribution.
[b] Adjusted for Neu.
* $p < 0.05$ vs. control group.
** $p < 0.05$ vs. other groups.
*** $p < 0.05$ vs. mild group.
**** $p < 0.05$ vs. moderate group.

Table 2
Pearson correlation coefficients between LT production/hsCRP and confounders.[a]

Variables	$LnLTB_4$		LnCysLTs		LnCRP	
	r	*p*	*r*	*p*	*r*	*p*
Age	0.119	NS	0.041	NS	0.098	NS
Gender	−0.086	NS	−0.112	NS	0.009	NS
BMI z-score	0.358	<0.001	0.383	<0.001	0.450	<0.001
Disease duration	0.042	NS	−0.067	NS	−0.057	NS
Adenotonsillar size	0.146	NS	0.063	NS	0.152	NS
SBP[a]	0.222	0.001	0.214	0.002	0.157	0.023
DBP[a]	0.211	0.002	0.177	0.011	0.165	0.017
MAP[a]	0.229	0.001	0.204	0.003	0.171	0.013
Glucose	−0.052	NS	−0.086	NS	−0.102	NS
Ln(Triglyceride)	0.027	NS	0.073	NS	0.113	NS
Total cholesterol	0.126	NS	0.093	NS	0.135	NS
HDL	0.004	NS	−0.076	NS	−0.111	NS
LDL	0.131	NS	0.119	NS	0.185	0.007

[a] Adjusted for age, sex and BMI z-score.

3.4. Regression analysis

Stepwise multiple regression analyses were performed using $lnLTB_4$ and lnCysLTs as the dependent variables, with age, sex, adenotonsillar size, BMI z-score, Neu, Lym, Mon, glucose, total cholesterol, ln(Triglyceride), and lnAHI as covariates. The independent predictors of $lnLTB_4$ and lnCysLTs both included lnAHI ($p < 0.001$) and BMI z-score ($p < 0.001$, adjusted $R^2 = 0.253$ and 0.275 respectively) (Table 4).

$$lnLTB_4 = 4.340 + 0.208 \times lnAHI + 0.119 \times \text{BMI z-score}$$

$$lnCysLTs = 5.342 + 0.221 \times lnAHI + 0.142 \times \text{BMI z-score}$$

4. Discussion

The present study demonstrates that children with SDB have a significantly greater systemic inflammatory response as reflected by increased level of LT concentration, leukocyte (major Neu) and hsCRP level (Table 1). Moreover, all of these inflammatory markers were elevated in the children with more severe SDB, suggesting that the magnitude of inflammation is related to the severity and frequency of upper-airway obstructive episodes during sleep.

Several investigators have tried to assess inflammatory measures in children with SDB. Gozal et al. [23] reported the elevation in levels of proinflammatory cytokine interleukin 6 (IL-6) and the decrease in anti-inflammatory cytokine IL-10 in nonobese children with SDB. Tam et al. [24] demonstrated that even mild cases had significantly elevated levels of interferon-gamma and a trend toward elevated IL-8 levels. Kaditis et al. [25] showed that fibrinogen values were higher in snoring children. And O'Brien et al. [26] also found elevated levels of plasma

Table 3
Partial correlation coefficients among systemic inflammatory markers.[a]

Variables	$LnLTB_4$		LnCysLTs		LnCRP	
	r	*p*	*r*	*p*	*r*	*p*
$LnLTB_4$	–	–	0.457	<0.001	0.343	<0.001
LnCysLTs	0.457	<0.001	–	–	0.370	<0.001
LnCRP	0.343	<0.001	0.370	<0.001	–	–
WBC	0.203	0.003	0.132	NS	0.122	NS
Neu	0.299	<0.001	0.202	0.003	0.261	<0.001
Lym	−0.085	NS	−0.130	NS	−0.208	0.003
Mon	0.094	NS	0.187	0.007	0.149	0.031
LnEos	−0.112	NS	−0.083	NS	−0.059	NS
LnBas	−0.084	NS	−0.115	NS	−0.178	0.010

[a] Adjusted for age, sex and BMI z-score.

Table 4
Stepwise multiple regression models of lnLTs/lnCRP[a].

	Constant		LnAHI		BMI z-score		Adjusted R^2
	B	*p*	*B*	*p*	*B*	*p*	
$LnLTB_4$	4.340	<0.001	0.208	0.001	0.119	0.001	0.253
LnCysLTs	5.342	<0.001	0.221	0.001	0.142	0.001	0.275

[a] Independent variables considered: age, sex, adenotonsillar size, BMI z-score, Neu, Lym, Mon, glucose, total cholesterol, ln(Triglyceride) and lnAHI.

adhesion molecules. In this study, our results for serum LTB_4 concentration in SDB are compatible with Lefebvre et al.'s [27] findings in adults. No other measurements of both LTB_4 and cysLTs have been reported in the peripheral blood of adults or children with SDB. This study demonstrates that LTB_4/cysLT concentrations significantly correlate with PSG parameters (AHI, OAI, ODI, SLT90%, and mean and minimal SpO_2) (Table 1), and that the main determinant of LT production is AHI (Table 4), suggesting that long-lasting intermittent hypoxia may play a major role in the activation of the 5-LO pathway. The mechanism may involve the formation of reactive oxygen species [28,29], which are the greatest contributors to the generation of adhesion molecules and the production of leukocytes and LTs [30]. Of note, hypoxia cannot explain the entire association between AHI and the production of LTs. AHI contains additional information about frequency and pattern (intermittent falls) of desaturation, and other associated physiological stressors (airway obstruction and sleep fragmentation) which are also relevant to chronic systemic inflammation.

Our findings also show that the circulating neutrophils are remarkably increased and the degree of neutrophilic inflammation correlates significantly and positively with LT production in children with SDB (Tables 1 and 3). In addition, erythrocyte, hemoglobin and platelet counts were the same in each group, which excluded the possibility that the increased neutrophils were only adaptive responses to stress reaction. Neutrophils are the most abundant population of leukocytes, which not only constitute the defense against pathogens but also can cause extensive tissue damage. The homeostasis of neutrophils in the circulation is regulated by a complex network of intracellular apoptotic/survival signaling pathways [10]. Previous studies have reported that the intermittent hypoxia/reoxygenation in SDB could delay or inhibit the apoptosis of neutrophils [11], while LTB_4 could induce a positive feedback autocrine pathway that supports neutrophil survival [31], which both have explained how the high percentage of neutrophils accumulates in the circulation of SDB children with the help of LTs. Of interest, Li et al. [32] found increased neutrophils in induced sputum collected from children with SDB, and the degree of neutrophilic inflammation correlated with the severity of SDB. In addition, LTB_4 is a potent chemoattractant that facilitates recruitment and endothelial cell adhesion of neutrophils to the inflammatory site and promotes recruitment of inflammatory cells into tissues [27]. This implies a probable existence of systemic neutrophlic inflammation in childhood SDB, and the elevations of neutrophils in the local airway as reported by Li et al. may be due to a persistent and enhanced neutrophil migration from the peripheral blood through the recruitment of LTs. Therefore, our findings confirm that systemic low-grade inflammation may either initiate or maintain the localized upper-airway inflammatory process in children with SDB. LTs are a family of biologically active compounds synthesized from the metabolism of arachidonic acid by a variety of cells, including neutrophils, monocytes/macrophages, eosinophils and mast cells [8,9]. And the amounts of LTB_4 and cysLTs depend on the distal enzymes LTA_4 hydrolase and LTC_4 synthase, respectively. It is difficult to determine whether the enhanced serum leukocytes are a cause, a consequence or both of the higher LT production in childhood SDB, because most of the inflammatory cells have the capacity to produce LTs, and the mechanisms responsible for inflammatory cell recruitment are not completely understood [9].

HsCRP is an important marker of inflammation, however the association between hsCRP and SDB severity has not been determined in children. Our findings of elevated hsCRP levels in SDB children are partly in agreement with four published pediatric reports [33–36]. However, Kaditis et al. [37] and Tam et al. [24] found no statistical differences in hsCRP values between SDB groups and controls, and no correlation between hsCRP and any PSG parameters. Although the role of hsCRP in childhood SDB remains in dispute, most researchers agree that the discrepancy among reports may be because of the subjects' ages and degree of obesity [36,37]. This implies that shorter disease durations and lower adipose states are connected with lower inflammatory levels, including no inflammation whatsoever. However, this was not the case in our report. Although our subjects were much younger and leaner (mean age [range] was 5.3 [2.0–9.0] years, BMI z-score was 0.76 [−2.01 to 6.52]) than the children in all previous studies, there was still an obvious elevation of hsCRP in the SDB groups. Furthermore, even the mild cases had higher hsCRP than the controls, which implies that the inflammatory process may occur earlier in our population. The correlation of hsCRP with metabolic variables was not significant (except for LDL) (Table 2), which also does not accord with previous reports. The children in our study were younger and thus may have had less exposure to metabolic syndrome. In addition the prevalence of childhood obesity is lower in China than in the United States or Europe. So ethnicity, environment and life-style may be important contributors to discrepancies found in the association of hsCRP and SDB from different areas. HsCRP is a circulating marker of cardiovascular risk [13,14], and neurocognitive morbidity in children [35], and we found a significant correlation between LTs and hsCRP (Table 3), thus the clinical significance of persistently raised LTs and its influence on end-organ morbidity should be a focus of future work in this area. This report extends the present knowledge by providing evidence that SDB may induce potential systemic complications much earlier in life and disease stage, at least among Chinese children.

Possible limitations of the present study would be that the PSG and nasopharyngoscopy were not performed on the control subjects due to the objections of their parents and that the evaluation of adenoid and tonsil size was not conducted by a single designated investigator, which undoubtedly could cause large inter-individual variability. Finally, the selection of subjects with a wider range of age and disorder severity may reduce the impact of confounders on survival biases.

In conclusion, this investigation demonstrates the presence of systemic inflammation characterized by marked increases in LTB_4/cysLTs, suggesting that the 5-LO pathway may participate in the pathogenesis of SDB in childhood. The disease severity as reflected by AHI, and to a lesser extent obesity, are both determinants of LT production. LT level has an intensive association with circulating Neu count and hsCRP. Further longitudinal studies are needed to confirm our findings, to demonstrate the clinical significance of persistently raised LTs and its influence on end-organ morbidity, and to investigate the efficacy of anti-leukotriene medicines in the treatment of children with SDB.

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